

## I. AMENDMENTS

### IN THE SPECIFICATION

**Please replace the seventh full paragraph on page 7 with the following rewritten paragraph:**

This object is solved by the recombinant manufacturing process set out in Claim 1 process for preparing of the complete gp190/MSP1 protein of Plasmodium, in particular Plasmodium falciparum characterized in that the complete gene for gp190/MSP1 is expressed in a suitable system, preferably a host organism, by which a complete gp190/MSP1 gene and the protein coded by it are obtainable in sufficient quantities.

**Please replace the fifth full paragraph on page 10 with the following rewritten paragraph:**

This object is solved by the invention named in Claim 17 complete DNA sequence, suitable for expression, of the gp190/MSP1 surface protein of Plasmodium, in particular P. falciparum, preferably obtainable through the recombinant process for preparing of the complete gp190/MSP1 protein of Plasmodium, in particular Plasmodium falciparum characterized in that the complete gene for gp190/MSP1 is expressed in a suitable system, preferably a host organism by which the sequence can be obtained by the recombinant manufacturing process described above.

**Please replace the second full paragraph on page 14 with the following rewritten paragraph:**

Fig. 3C-~~3X~~: Total sequence of gp190<sup>S</sup>

**Please replace the third full paragraph on page 14 with the following rewritten paragraph:**

Fig. 3D-~~3Y~~: N- and C-termini of gp190<sup>S1</sup> variant

**Please delete the seventh full paragraph on page 14.**

**Please replace the eighth full paragraph on page 14 with the following rewritten paragraph:**

Fig ~~5B5C~~: Electrophoretic characterization of gp190<sup>S1</sup> purified from HeLa cells.

**Please delete the tenth full paragraph on page 14.**

**Please replace the eleventh full paragraph on page 14 with the following rewritten paragraph:**

Fig ~~6B6C~~: Polyacrylamide gel electrophoresis of gp190 from T. gondii.

**Please replace the second heading on page 16 with the following rewritten paragraph:**

C. Total sequence of gp190<sup>S</sup> (see Fig.3C-3X)

**Please replace the third heading on page 16 with the following rewritten paragraph:**

D. N- and C-termini of gp190<sup>S1</sup> variant (see Fig. 3D3Y)

**Please replace the fourth heading on page 17 with the following rewritten paragraph:**

Example. 3: Tetracycline-controlled expression of gp190<sup>S1</sup> in HeLa and CHO cells and isolation of the product (see also Fig. 5 and 6b6e)

**Please replace the first full paragraph on page 18 with the following rewritten paragraph:**

The production of luciferase (left), gp190<sup>S1</sup> (middle) in the absence of Tc was demonstrated in HITA93-9 cells, which contain the bidirectional transcription unit of (A). Following addition of Tc no noteworthy synthesis of gp190<sup>S1</sup> was shown (as represented in Fig. 5B, right).

**Please replace the second full paragraph on page 18 with the following rewritten paragraph:**

The HeLa cell clone HtTA93-9 as well as the CHO cell clone CHO27-29 have been cultivated with or without Tc. Cell extracts separated by electrophoresis have been analyzed with mAK5.2 by means of "Western blotting" (Fig. ~~5B5C~~); analysis of the CHO cell line is shown on the left, of HeLa on the right. (1) = culture without, (2) = culture with Tc, (3) = non-transfected HtTA-1 cell line. Molecular weight standards are in each case indicated on the left.

**Please replace the fourth full paragraph on page 18 with the following rewritten paragraph:**

The polyacrylamide gel stained with Coomassie (Fig. ~~6B6C~~) following electrophoresis displayed a product consisting of gp190<sup>S1</sup> as well as another protein of about 50 kD. The latter was not derived from gp190<sup>S1</sup> and thus originated from the HeLa cells. Its projected removal should nevertheless present no difficulty in principle.

**Please replace the first full paragraph on page 19 with the following rewritten paragraph:**

Transfection of *T. gondii* with pTT190 led to the isolation of parasite lines which expressed constitutively gp190<sup>S</sup>. Immunofluorescence with mAK5.2 (~~middle picture~~) showed not only expression of the gene but also situated the binding of the expression product close to the surface of the parasite, since it, like SAG-1, provokes the same pattern of immunofluorescence (~~right section of fig. 6B~~). ~~On the left in Fig. 6B a phase-contrast photograph of the middle picture is shown.~~

**Please replace the second full paragraph on page 19 with the following rewritten paragraph:**

By means of affinity chromatography (mAK5.2 column) gp190<sup>S</sup> was purified from a prepared quantity of *T. gondii* ( $5 \times 10^9$  parasites). The extremely pure protein possessed the anticipated molecular weight, as the Coomassie-stained polyacrylamide gel indicated following electrophoresis (2-3 on Figure ~~6B6C~~). At no. (1) on Fig. 6C purified gp190<sup>S1</sup> from CHO cells is represented with molecular weight marked on the left side.

**Please replace the first full paragraph on page 21 with the following rewritten paragraph:**

In the construct pBi5-gp190<sup>S1</sup> a bidirectional promoter was activated by the Tc-controlled transcription activator and initiated transcription of both gp190<sup>S1</sup> and the luciferase indicator gene. In the presence of Tc the promoter is inactive. The transcription unit was transferred into both HeLa and CHO cells, which both synthesize constitutively tTA (HtTA line: Gossen, M. and Bujard, H. (1992), Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc. Natl. Acad. Sci. USA* 89, 5547-5551; CHO-tTA line, unpublished).

Through cotransfection ( $\text{Ca}^{2+}$ -phosphate method) with a hygromycin-resistance-inducing marker gene was selected for successful chromosomal integration. Hygromycin-resistant clones were then investigated for regularity of the expression >Tc, in which luciferase activity was used as indicator. The gp190 synthesis was tested in well regulable clones (regulation factor •Tc 1000). Immunofluorescence analysis (~~Fig. 5B~~) as well as investigation by "Western blot" (Fig. ~~5B5C~~) allowed the identification in both cell types of clones which synthesized gp190 under strictly regulable conditions. The best regulable of 20 clones were in each case subcloned. The subclones HtTA93-9 and CHO27-29 were used for cultures on a scale of 10:1. From cell extracts of these cultures intact gp190<sup>S1</sup> could be isolated by means of affinity chromatography (mAK5.2). The material was homogeneous except for a single cellular component which did not derive from gp190<sup>S1</sup> and made up 25% of the preparation (Fig. ~~6B6C~~). It had to be removed in a further purification step.

**Please replace the third full paragraph on page 21 with the following rewritten paragraph:**

This expression construct was transfected into *T. gondii*. Selection with chloramphenicol led to resistant clones synthesizing gp190 which were detected by immunofluorescence (~~Fig. 6B~~).

**Please replace the first full paragraph on page 22 with the following rewritten paragraph:**

The immunofluorescence with anti-gp190 antibodies was indistinguishable from a corresponding pigmentation of the parasites by means of antibodies against SAG1, the main surface protein of *T. gondii*. It may be deduced from this that gp190 is bound to the surface of *T. gondii*. Several *T. gondii* clones (Nos. 3.1 to 3.4) were characterized and saved for the production of gp190. Using affinity chromatography (mAK5.2) gp190 was isolated from *T. gondii* cultures (clone 3.4) cultivated on a preparative scale. Electrophoretic analysis revealed a homogeneous product with a migration rate similar to that of the intact protein (Fig. ~~6B6C~~).